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Intrauterine infusion of BQ-610, an endothelin type A receptor antagonist, delays luteolysis in dairy heifers

Christopher S. Keator*, David T. Schreiber, Thomas A. Hoagland, John A. McCracken, Robert A. Milvae

Department of Animal Science, University of Connecticut, 3636 Horsebarn Hill Road Extension, Storrs, CT 06269-4040, United States

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Abstract

Three separate *in vivo* experiments were conducted to evaluate the putative role of endothelin-1 (ET-1) during luteal regression in heifers. In Experiment 1, a single intraluteal injection of 500 μg BQ-610 [(*N*,*N*-hexamethylene) carbamoyl-Leu-D-Trp (CHO)-D-Trp], a highly specific endothelin A (ET_A) receptor antagonist, did not diminish the decline in plasma progesterone following a single exogenous injection of 25 mg prostaglandin F2 alpha ($PGF_{2\alpha}$) administered at midcycle of the estrous cycle. In Experiment 2, six intrauterine infusions of 500 μg BQ-610 given every 12 h on days 16–18 delayed spontaneous luteolysis, as evidenced by an extended elevation ($P = 0.054$) of plasma progesterone concentration. In Experiment 3, heifers were administered six intrauterine infusions of BQ-610 or saline on days 16–19, and peripheral blood samples were collected from day 11 to 16 (before infusion), hourly on days 16–19 (during infusion), and on days 20–25 (after infusion). BQ-610 treated heifers had markedly higher ($P < 0.0001$) levels of plasma progesterone compared with saline controls, and this effect was most notable during the infusion period (treatment by period interaction; $P \leq 0.05$). Heifers infused with BQ-610 also had higher progesterone levels on day 21 (treatment by time interaction; $P \leq 0.05$). Mean plasma concentrations of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM), the primary metabolite of $PGF_{2\alpha}$, were measured in the samples collected hourly and were not different ($P \geq 0.05$) between treatments. These results indicate that the *in vivo* antagonism of the ET_A receptor can delay functional luteolysis, and supports the theory that ET-1 regulates luteal function in ruminants.

Keywords: Corpus luteum function; Progesterone; Endothelin; Endothelin type A receptor; Prostaglandin F2 alpha

1. Introduction

Prostaglandin F2 alpha ($PGF_{2\alpha}$) pulses of uterine origin are recognized as the luteolytic signal in domesticated ruminants [1,2], but the mechanisms by which $PGF_{2\alpha}$ manifests luteal regression remain unclear [3]. Circulating concentrations of $PGF_{2\alpha}$ [3], endothelin [4,5], angiotensin II [6] and nitric oxide [7] all increase during bovine luteolysis. The relationship between endothelin-1 (ET-1) and $PGF_{2\alpha}$ has been examined in a number of reproductive tissues, including the endometrium [8], myometrium [9], oviduct [10], follicles [11] and the corpus luteum [12].

Substantial evidence exists which suggests that ET-1, first isolated from porcine aortic endothelial cells [13] and the most potent mammalian vasoconstrictor discovered [14], may mediate the luteolytic action of $PGF_{2\alpha}$ in ruminants. Luteal ET-1 mRNA content rises throughout the bovine estrous cycle [15] and increases after exposure to $PGF_{2\alpha}$ in both cows [15] and sheep [16]. The

* Corresponding author at: Division of Reproductive Sciences, Oregon National Primate Research Center, 505 NW 185th Avenue, Beaverton, OR 97006, United States. Tel.: +1 503 614 3780; fax: +1 503 690 5563.
E-mail address: keatorc@ohsu.edu (C.S. Keator).

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addition of ET-1 to dispersed luteal cells inhibited progesterone production, and the effects were negated by the addition of the ET_A receptor antagonists BQ-123 or BQ-610, in the sheep [16] and cow [17], respectively. The luteolytic effect of ET-1 is amplified in microdialyzed bovine corpora lutea (CL) after prior exposure to $PGF_{2\alpha}$ [18], and sheep administered sub-luteolytic doses of $PGF_{2\alpha}$ followed by injections of 100 μg ET-1 exhibited a pronounced decrease in plasma concentrations of progesterone with shortened estrous cycles [16].

ET-1 elicits physiological responses via two G-protein coupled receptor subtypes and both the subtype A (ET_A) [17] and subtype B (ET_B) [19,20] receptors have been detected in the bovine CL. Girsh et al. [17] demonstrated that BQ-610, an ET_A receptor antagonist, inhibited the actions of ET-1 in dispersed luteal cells and tissue slices. In the bovine uterus, endothelin receptor profiling has not been determined, although both receptor subtypes have been isolated and characterized in the bovine oviduct [21] and in the uterus of humans [22] and sheep [23].

Although strong supportive evidence exists for a role of ET-1 during luteolysis, the inability of ET-1 – in the absence of $PGF_{2\alpha}$ – to induce luteolysis in domesticated ruminants is paradoxical. Therefore, to try and elucidate a better understanding of the physiological role of ET-1 during functional luteolysis, we designed three *in vivo* experiments which tested the hypothesis that ET-1 acts through the ET_A receptor to regulate $PGF_{2\alpha}$-induced and natural luteolysis. Our objectives were to administer BQ-610 to heifers, which is a highly selective ET_A receptor antagonist, and then measure changes in the peripheral plasma concentrations of progesterone and 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM), the primary metabolite of $PGF_{2\alpha}$. We report novel findings which suggest that ET-1, via the ET_A receptors, directly modulates progesterone production without altering the uterine secretion/production of $PGF_{2\alpha}$.

2. Materials and methods

2.1. Animals

Normally cycling nulliparous Holstein heifers were group housed at the University's Cattle Resource Unit. Estrous cycle activity was monitored and recorded twice daily, and animals were determined to be in estrus (designated as day 0) when heifers stood to be mounted. All surgical procedures, subsequent treatments and post-operative care were performed at the Kellogg Dairy Center's surgical facility. All experimental procedures followed the guidelines stipulated by the University of Connecticut's Institutional Animal Care and Use Committee.

2.2. Experiment 1: intraluteal injection

Heifers were randomly assigned to the BQ-610 treatment group ($n = 4$) or the saline control group ($n = 4$). On day 9–11 of the estrous cycle, animals were palpated rectally to ensure the presence of a single CL. An Abbocath-T (16 ga. by 140 mm) catheter (Abbott Laboratories, North Chicago, IL) was then inserted into the right jugular vein and a 10 ml blood sample was withdrawn. Heifers were given a local anesthetic of 2% lidocaine HCl (Phoenix Pharmaceutical Inc., St. Joseph, MO) between the coccygeal vertebrae, and the CL was then visualized with a Pie Medical ultrasound unit equipped with a 5 Mhz transducer (Pie Medical Equipment BV, Maastricht, The Netherlands). A modified 18 ga. 'shorty' direct-contact ovum pick-up needle (with echo tip), attached to a single line ovum pick-up tubing set (Cook Veterinary Products, Bloomington, IN) was fitted with a 1.0 ml tuberculin syringe (Becton Dickinson and Company, Franklin Lakes, NJ). The transducer was inserted into the vagina as far as possible with the transducer head in a position either to the left or the right of the external os cervix, and the CL was manually aligned with the puncture trajectory. The needle tip was then manually guided and inserted into the approximate center of the CL, which was visualized on the ultrasound monitor. Treatment solutions of either 500 μl saline or 500 μl saline containing 500 μg BQ-610 (Peninsula Laboratories Inc., A Member of the Bachem Group, San Carlos, CA) were then injected directly into the CL, which was also confirmed visually on the ultrasound monitor. After 15 min the first blood sample was collected and 30 min after BQ injection, 25 mg $PGF_{2\alpha}$ (Lutalyse; Pfizer, New York, NY) was injected intramuscularly (i.m.) into the gluteal muscles ipsilateral to the CL-bearing ovary. Jugular venous blood samples were withdrawn at: 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24, and 36 h post $PGF_{2\alpha}$ injection. After the final blood sample, animals were grouped housed and then monitored three times a day for standing estrus.

2.3. Experiment 2: intrauterine infusions on days 15–17 and 16–18

Heifers ($n = 9$) were randomly selected and assigned to one of the following three treatment groups ($n = 3$/group): saline, BQ-610 infused on cycle days 15–17 (BQ17) and BQ-610 infused on cycle days 16–18 (BQ18). On day 13 of the cycle, animals were palpated